EXPERIMENTAL RESEARCHES IN INFECTIVE EMBRYONAL PATHOLOGY.[1]

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TWO facts have induced me to make these researches: First, I wished to know if an embryonal tissue in contact with a certain virus has the same strength of resistance as the tissue of an adult. We can get a clear idea of all these relations only when we shall have studied also all the relations in the embryonal life. Therefore, after the comparative studies of Metschnikoff, I believe that it will be necessary to try, also, the study of the infective pathology of the embryo. The second reason for making these studies was the fact that all who have occupied themselves in the study of infective embryonal pathology have either not found any micro-organism in the tissues of the embryo, while they were present in the tissue of the mother, or they have found those micro-organisms not very numerous. Therefore most inquirers have believed that the last fact could be the effect of the difficulty with which the micro-organisms would pass from the mother to the fœtus. But we must pay regard to the power of the organisms to resist the microbes. And about this fact we possess only the indirect observations of Baumgarten.

The opinion of those who believe that the placenta is a filter, which may be penetrated if it is altered, does not answer the other question, What becomes of the micro-organisms which pass through the placenta and come in contact with the tissues of the embryo?

All inquirers admit the presence of germs in the sperma of animals taken ill by certain infections, as tuberculosis. Notwithstanding one does not admit the possibility that the tuberculous father can generate a tuberculous child, it is taken for granted that a syphilitic father can generate a syphilitic child. Why shall we make such a difference for these two infections? While there have not been found tubercles or bacilli in a fœtus generated by a tuberculous father, specific lesions have been found in the fœti of syphilitic parents. And here we can ask if the egg does not receive the bacilli with the spermatozoid, or are the tissues of the embryo resisting to the development of the bacillus? Therefore I chose the eggs of the hen, which I could study with great facility in the different brood periods. I have inoculated different microbes into the eggs of the hen before placing them in the brood-oven, and at different periods of the brood period I have inoculated also the albumen which encloses the embryo. The albumen which has been fecundated and bred is very favorable to the development of a certain series of pathogenic microorganisms, but the albumen which has not been bred does not have this power.

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Here I may add that the reception of the microbes from the albumen by the embryo does not happen by the vascular layer, but by the allantoid bladder, and therefore the inoculated microbes penetrate to the tissue of the embryo only by the tenth day. The microbes I inoculated were those of anthrax bacilli and spores of the cholera of fowls, of the pneumococcus of Friedländer, of the fowl tuberculosis, of the tuberculosis of the mammalia, and the toxic products of the fowl tuberculosis and that of the mammalia.

The following method I have chosen to prove the presence and the phases of the above-named microbes: I made the histological examination of the albumen at different brood periods, and the histological examination of the different tissues of the embryo (without the bile and the contents of the stomach). And then I made cultures from all these parts respectively, and inoculated animals with all the matters, which I examined.

Of the organs of the embryo the most interesting was the liver, which one can comprehend, since it is the first entrance from the albumen into the embryo. At the same time I studied the infected embryos, which had been brooded and kept in life for a long time, that I might observe in what manner the microbes absorbed in the embryonal life would behave in the life out of the egg. The following results I have obtained:

(1) The albumen, fecundated and brooded, taken from the eggs in different brood periods, is a favorable medium for the growth of the above-named micro-organisms. But the same fecundated and brooded albumen does not induce the growth of these microbes in the living embryo.

(2) During the life of the embryo, the microbes, which are present in its tissues, do not multiply.

(3) The tissues of the living embryo can contain many microbes which are capable of killing the control animal, whilst the embryo does not suffer from the presence of those microbes.

(4) The microbes can be destroyed or attenuated in the tissues of the embryo, but not in the surrounding albumen.

(5) Some embryos can die under the action of the pathogenic microbes of the adult fowl.

(6) The embryos can be brooded, and some of them die after a few days by infection; the others remain alive, because the microbes are destroyed completely; but generally they are marasmic ones.

(7) The infection can develop much time after brooding as a chronic infection (tuberculosis of the fowl).

(8) The tuberculosis of the fowl appears first in the liver, where it can heal, and later in the lungs and spleen.

(9) When the embryo has destroyed the bacillus of the fowl tuberculosis, it is born marasmic, and with this form after a long time can die without tuberculosis in the organs.

(10 The same effect of marasmus one can obtain by inoculating into the eggs dead bacilli of the fowl tuberculosis.

(11) The embryo, having destroyed the bacillus of the fowl tuberculosis and been born marasmic, can grow very well.

(12) The destruction of the virus by the embryo does not make it refractory against the same infection when it was inoculated a long time after hatching (fowl cholera).

(13) If the embryo does not destroy the bacillus of the tuberculosis of the mammalia, this is not transformed into that of the fowls.

(14) The embryos which have been inoculated with the pathogenic microbes of the adult fowl resist the infection until the microbes have entered in great quantity into the tissues (anthrax, borbone, pneumobacillus).

(15) The liver is an organ where the microbes are destroyed, but often the virus is already attenuated (cholera of the fowl).

(16) Whilst all these changes happen in the tissue of the embryo, the rest of the microbes remaining in the albumen maintain their pathogenic power. Therefore the animals inoculated with albumen die, but those which are inoculated with the organs of the embryo and with the cultures of these organs do not die.

(17) The attenuation of the virus (cholera of the fowl) does not happen within a fortnight during the brooding time. On the contrary, the virus is virulent in the albumen and in the tissues of the embryo. The attenuation appears on the eighteenth day of the brooding time.

(18) The attenuated virus remains for a few days after the birth in the pullet, but disappears with the growth.

Having made the experiments with the embryos, I wished to study the manner of reaction in an embryo of a mammalia, and I chose the embryo of the rabbit and the virus of tuberculosis.

The following method has been used:

I inoculated buck conies by the jugular vein with the tuberculosis of the mammalia, and I examined their semen from twenty-four hours up to three months thereafter.

It was difficult to detect the bacillus of tuberculosis in the semen during the first days. On the contrary, I found from the twenty-fifth day until three months a great number of bacilli. I inoculated with the semen sea-hogs, which became tubercular.

Other buck conies, in whom I inoculated the same dose of tuberculosis in the jugular vein, have been put in contact with does which became pregnant. The young ones I maintained in part in life, other ones I took out of the uterus of the mother and inoculated their organs into sea-hogs.

Only two sea-hogs inoculated with organs of a fœtus of a tubercular father became tubercular.

Of the rabbits, some died spontaneously and others have been killed. These animals before two months after birth did not present tubercular lesions. From two months to a year, one could observe in the liver nodules which had the classical structure of tubercle, cheesy substance in the centre, epithelioid and giant-cells, and lymphoid infiltration on the periphery.

The most scrupulous researches, made after the method of Ehrlich, never have shown tubercle bacilli in these young forms of tubercle. Inoculating such nodules into sea-hogs, there was no effect to be seen, or they died by marasmus, but never by tuberculosis. Some of the mothers of these rabbits (of tubercular paternity) died tubercular by vaginitis tubercularis, and others by tuberculosis of the abdominal organs without vaginitis tubercularis. These mothers have become tubercular a long time after the delivery, and their young have been separated from them as soon as they could eat.

Another series of researches has been made with pregnant does, into whom has been inoculated in the jugular vein, from the fifteenth to the twenty-fifth day of the pregnancy, a culture of tuberculosis. Many of the does have been killed from one hour after the inoculation until fifteen days, and the organs of these fœti have been taken with the greatest circumspection, and the embryos have been placed for a long time in sublimate, and then were washed with alcohol and cut by sterilized instruments, and all their organs and the contents of the stomach have been inoculated into a series of sea-hogs. But from all these sea-hogs only three became tubercular, and just those which were inoculated with organs of fœti killed in the first forty-eight hours after the inoculation applied to the mother. All the other sea-hogs either died by marasmus, as if they had been inoculated with a culture of sterilized tuberculosis, or they did not feel anything.

All the rabbits born of mothers treated in that manner (by the inoculation of tuberculosis into the jugular vein) have shown nodules of tubercular structure in the liver, but never before the fourth month after the birth. These nodules presented at the first time central, cheesy masses, epithelioid and giant-cells around the cheesy mass, and lymphoid infiltration on the periphery. Inoculating these nodules into sea-hogs, these never became tubercular, but some of them died by marasmus, and the same nodules examined by the method of Ehrlich never showed the presence of bacilli.

In a few cases I could still find nodules in the lungs with the same structure, but without bacilli. The nodules of the liver do not present a progressive but a regressive phase, so that some young of the same mother, killed after four or five months, present the most typical forms of the structure of the tubercle, while those killed after a year will show only the tuberculosis in the cicatrizing phase with little cheesy substance and with some giant-cells. The sea-hogs inoculated with these nodules did not become tubercular, but died of marked marasmus.

To remove any doubt that the nodules shown in the young of the tubercular mothers had been produced by nursing, I inoculated into the does, two days after the delivery, a strong dose of tuberculosis in the jugular vein, but those does never have borne young with tuberculosis in the liver and in the lungs.

Therefore, for the embryo of the mammalia (rabbit) I can give thus far the following conclusions:

(1) The fœtuses of mothers grown tubercular during the pregnancy can contain the bacillus after four hours from the inoculation in the jugular vein of the mother.

(2) In the placenta there is no development of tuberculosis after fifteen days from the moment that the bacillus is found in the blood of the mother; also it is difficult to find the bacilli in the vessels of the placenta.

(3) The organs of the fœtuses of mothers grown tubercular can render tubercular sea-hogs in the first forty-eight hours after the inoculation, because they contain living, though few bacilli.

(4) The great mass of sea-hogs not rendered tubercular (inoculated with the organs of a fœtus) died by marasmus as if they had been inoculated with dead bacilli.

(5) The rabbits born from tubercular mothers do not present tubercles until the sixth month after their birth, but after this

period they can contain tubercles in the liver and in the lungs, but without bacilli visible to us.

(6) In an exceptional case I could find that the organs of a fœtus from a tubercular father, inoculated after its birth into a sea-hog, made it tubercular.

(7) The young from a tubercular father present only three months after the birth tubercles in the organs, but without bacilli visible to us.

REMARKS.

How may be explained these facts found in the mammalia? If the lesions found by me in the liver of the rabbits coming from tubercular father and mother may be of bacillar origin, I believe that the history of the above-named facts in the fowl embryo can give us this interpretation; that the virus can be destroyed or attenuated by the embryonal powers; therefore, the tubercular bacilli, brought into contact with the embryonal tissues of the mammalia, have been paralyzed or killed by these, and where the bacilli remained they caused a phlogosis with all the characters of the tubercle, as Prudden has observed in sterilized cultures of bacilli. Another fact supports my idea: the tubercles generated by dead bacilli undergo an involutive fibrous, not progressive phase.

The most destruction of the virus has been observed in the liver of the fowl embryo. Therefore, I can affirm that the liver is in embryonal life a great protector against the infections, being capable of destroying inoculated microbes, even the spores of anthrax.

GENERAL CONCLUSIONS.

During its life the embryo does not permit the growth of pathogenic microbes in its tissues (except only in an exceptional case), but it can destroy them, attenuate or accumulate them, and develop them later in the life out of the egg.